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Experimental Neurology 205 (2007) 536–546
Experimental Neurology
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# Striatal dopamine $D_2$ receptors attenuate neuropathic hypersensitivity in the rat

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Received 30 January 2007; revised 13 March 2007; accepted 15 March 2007
Available online 21 March 2007

**Abstract**

Earlier studies indicate that striatal dopamine $D_2$ receptors are involved in pain regulation in non-neuropathic conditions. We assessed whether striatal dopamine $D_2$ receptors contribute to pain regulation also in neuropathic conditions. The spared nerve injury model of neuropathy was induced by unilateral ligation of the tibial and common peroneal nerves in the rat. In awake nerve-injured animals, pain-related withdrawal responses to calibrated monofilaments or noxious heating were attenuated following striatal administration of a dopamine $D_2$ receptor agonist quinpirole. Pain-related responses were attenuated only in the nerve-injured limb ipsilateral to the injection and in the midline (tail). In unoperated controls, striatal administration of quinpirole at an antihypersensitive dose did not influence withdrawal responses to mechanical stimulation. Attenuation of pain-related responses induced by striatal administration of quinpirole was reversed by intrathecal administration of a dopamine $D_2$ receptor antagonist (eticlopride) or a non-selective 5-HT receptor antagonist (methysergide), but not by an $\alpha_2$-adrenoceptor antagonist (atipamezole). In the rostroventromedial medulla of lightly anesthetized neuropathic animals, striatal administration of quinpirole significantly decreased the activity of presumably pronociceptive cells that are activated by noxious stimulation. The innocuous H-reflex in lightly anesthetized control animals was not suppressed by striatal administration of quinpirole at an antihypersensitive dose. The results indicate that striatal dopamine $D_2$ receptors attenuate neuropathic hypersensitivity. The antihypersensitive effect induced by striatal dopamine $D_2$ receptors in peripheral neuropathy involves suppression of impulse discharge of presumably pronociceptive neurons in the rostroventromedial medulla, and a descending influence acting on spinal 5-HT and dopamine $D_2$ receptors.


*Keywords:* Descending inhibition; Dopamine $D_2$ receptor; Neuropathic pain; Rostroventromedial medulla; Serotonin receptor; Striatum

## Introduction

There is accumulating evidence indicating that the striatum, the main input nucleus of the basal ganglia, might have a role in pain processing (for reviews see Chudler and Dong, 1995; Hagelberg et al., 2004; Neugebauer, 2006). This is indicated by the findings that striatal neurons respond to noxious stimulation in experimental animals (e.g., Chudler, 1998; Chudler et al., 1993) and painful stimulation increases striatal blood flow in human subjects (Casey et al., 1996; Coghill et al., 1999, 2002; Derbyshire et al., 1997; Iadarola et al., 1998; Jones et al., 1991; Svensson et al., 1997). Also, electrical stimulation of the striatum attenuates pain-related responses in nonhuman primates (Lineberry and Vierck, 1975). Although basal ganglia activation by painful stimulation is often attributed to the inhibition or preparation of motor activity, it has also been proposed to represent an engagement of cerebral attentional systems during sustained neuralgic pain (Downar et al., 2003). Dopaminergic innervation of the striatum from the substantia nigra and striatal dopamine $D_2$ receptors appear to have an important role in striatal processing of pain as indicated by the following findings. Dopaminergic nigrostriatal neurons were found to be activated by noxious stimulation (Schultz and Romo, 1987), lesions of nigrostriatal neurons enhanced nociception (Carey, 1986; Lin et al., 1981, 1984; Saade et al., 1997; Takeda et al., 2005) and striatal administration of dopamine $D_2$ receptor agonists suppressed pain-related responses in experimental animals

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doi:10.1016/j.expneurol.2007.03.010

(Lin et al., 1981; Magnusson and Fisher, 2000). In line with this, pain is a frequent symptom in degenerative diseases of the nigrostriatal dopaminergic system such as Parkinson's disease and burning mouth (Ford, 1998; Hagelberg et al., 2003b; Jääskeläinen et al., 2001; Schott, 1985), patients with Parkinson's disease have lower pain thresholds than healthy controls (Brefel-Courbon et al., 2005; Djaldetti et al., 2004), high dopamine $D_2$ receptor availability in the putamen is associated with low pain sensitivity in healthy subjects (Hagelberg et al., 2002; Pertovaara et al., 2004; Scott et al., 2006), and patients with some chronic pain conditions exhibit higher dopamine $D_2$ receptor availability in the putamen than their age- and sex-matched controls (Hagelberg et al., 2003a,b).

Peripheral nerve injury may produce long-lasting neuropathic pain and hypersensitivity. The maintenance of these nerve injury-induced symptoms depends on abnormal discharge from peripheral nerves (Devor, 2006) and pronociceptive changes in the spinal segmental mechanisms mediating and modulating pain-related signals (Woolf and Salter, 2006). Additionally, changes in descending pain modulation contribute to neuropathic symptoms (Pertovaara, 2000; Porreca et al., 2002). Because the striatum modulates spinal nociception (Belforte and Pazo, 2005) and reflexes through the superior colliculus and the nucleus raphe magnus (Basso and Evinger, 1996; Basso et al., 1996), we propose a hypothesis that the striatum and its dopamine $D_2$ receptors influence neuropathic symptoms by acting on the brainstem–spinal pathways regulating nociception. To test this hypothesis, we determined in animals with the spared nerve injury (SNI) model of neuropathy (Decosterd and Woolf, 2000) whether striatal administration of a selective dopamine $D_2$ receptor agonist modulates pain-related behavior and/or the discharge rate of pain regulatory neurons of the rostroventromedial medulla, a common final pathway for descending influence (Gebhart, 2004). Furthermore, various receptor antagonists were administered intrathecally to assess the role of spinal neurotransmitters involved in the possible modulation of pain-related behavior induced by striatal dopamine $D_2$ receptors.

## Materials and methods

### *Animals and drugs*

Adult male Hannover-Wistar rats (Harlan, Horst, The Netherlands) weighing 200–300 g were used in this study. They were kept in a room with a 12-h alternating light/dark cycle and had access to food and water *ad libitum*. They were also allowed some time (4–7 days) to get acclimatized to their new environment in the laboratory prior to the start of experiments. The study protocol was approved by the Institutional Ethics Committee of the University of Helsinki and the regional government of Southern Finland.

### *Surgical procedures for producing neuropathy*

For inducing neuropathy, the spared nerve injury (SNI) model, as described by Decosterd and Woolf (2000), was adopted. Prior to surgery, the rat was anaesthetized with sodium pentobarbital (OrionPharma, Espoo, Finland), administered intraperitoneally at the dose of 60 mg/kg. An incision was subsequently made into the skin on the lateral surface of the left thigh, followed by a section through the biceps femoris muscle to expose the sciatic nerve and its terminal branches: the sural, common peroneal and tibial nerves. The common peroneal and tibial nerves were then tightly ligated with 4–0 silk, sectioned distal to the ligation and 3–4 mm of the distal nerve stump removed. The sural nerve was left intact and care was exercised not to stretch it.

### *Procedures for striatal and intrathecal microinjections*

While the animals were under pentobarbital anesthesia and secured in a stereotaxic frame, a 26-gauge stainless guide cannula through which drugs could be delivered, was unilaterally and chronically implanted in the dorsolateral striatum (AP: 8.7 mm, ML: +4.2 mm, DV: −4.0 mm; Paxinos and Watson, 1998) of the rat, for intrastriatal drug administrations. Except for one group of rats, the guide cannula was implanted on the side ipsilateral to the injured (SNI) limb (left side) and anchored to the skull, using dental screw and cement. A stainless dummy cannula was inserted into the guide cannula to prevent blockage. This dummy cannula was always removed during test-drug administration. A 10-μl Hamilton microsyringe was connected to a 33-gauge stainless microinjection needle via a 30–40 cm non-toxic polyethylene with an outer diameter of 0.61 mm (PE-10, Becton Dickinson & Co. USA) tubing. Microinjection of drugs into the striatum was performed by lowering this 33-gauge injection needle through the guide cannula and delivering 0.5 μl of drug over a 10- to 30-s period. The injection needle protruded about 1 mm beyond the tip of the guide cannula. A deliberately trapped air bubble in the PE-10 tubing was used to monitor the flow of drug solution.

Intrathecal catheter was administered under pentobarbital anesthesia using a method described by Størkson and colleagues (1996). Briefly, a polyethylene (PE-10) tubing was inserted into the lumbar subarachnoid space for intrathecal drug administrations. The intrathecally inserted catheter was then fixed through a layer of superficial muscles, tunneled rostrally and made to appear through the skin in the occipital region. Upon recovery from anesthesia, 10–15 μl of 2% lidocaine hydrochloride (Orion Pharma), followed by 10–15 μl of saline was given through the catheter – with the help of a 50-μl Hamilton microsyringe – to verify if it was indeed intraspinally located. An immediate onset of a temporal hind limb paralysis (lasting 15–30 min) was considered to be indicative of correct intraspinal location of the catheter. Only rats with catheters located intraspinally and with no neurological deficits from the catheter were included in the study. Test-drugs were injected intrathecally using a 50-μl Hamilton microsyringe, flushed afterwards with saline.

### *Behavioral testing*

#### *Sensitivity to mechanical stimulation*

Mechanical hypersensitivity of the lateral plantar surface of the paw (sural nerve skin territory) was evaluated a day before

and again at various time points 2–4 weeks post SNI surgery. Both the ipsi- and contralateral paws were studied. For this, the rat was placed on an elevated wire grid and the lateral plantar surface of the paw was stimulated with five different calibrated von Frey monofilaments (North Coast Medical, Inc. Morgan Hill, CA, USA) producing following forces: 1.4 g, 2 g, 6 g, 10 g and 15 g. The monofilaments producing the lowest force should produce a rather selective activation of mechanoreceptors, while the monofilaments producing the highest force should activate also a population of nociceptors (Leem et al., 1993). At each particular time point studied, the paw ipsi- and contralateral to the nerve injury was stimulated repeatedly for five times with each of the five monofilaments applied at an ascending order. For each monofilament, the number of brisk withdrawal responses was recorded as a percentage of the five stimulations. Mean of the five resulting percentages for each limb was calculated and termed the pain score. Thus, if the animal responded to every presentation of every monofilament, it got the maximum pain score of 100.

*Noxious heat-evoked responses*

Radiant heat-induced limb withdrawal and tail-flick latencies were used to assess thermal nociception. For the limb withdrawal latency, Hargreaves' method (Hargreaves et al., 1988) was employed, using a radiant heat equipment (Ugo Basile's Plantar test model 7370, Comerio, Varese). For this, the rat was placed on a glass plate and radiant heat applied to the plantar surface of the hind paw from beneath, until spontaneous withdrawal of the hind paw was achieved. An electronic counter in the equipment registered the time in seconds from heat application to limb withdrawal and this was considered as the latency of limb withdrawal. The cut-off point was arbitrarily set at 15 s after which time heat application was discontinued to prevent possible tissue damage. For such a rat, the limb withdrawal latency was recorded as 15 s. For the tail-flick latency, radiant heat was applied to the tail, using a radiant heat equipment (Socrel model DS-20, Ugo Basile, Comerio, Varese, Italy). Time in seconds from the onset of tail heating to the first reflex tail-flick was registered by the equipment and taken to be the tail-flick latency. Ten seconds was arbitrarily set as the cut-off point beyond which heating was discontinued to prevent possible tissue damage. For both the limb withdrawal and tail-flick latencies, time points studied were: pre-drug, 1, 5, 15 and 30 min post drug. At each time point, data were collected in duplicate for the limb withdrawal and in triplicate for the tail-flick, averaged and expressed as percentages of pre-drug values. The minimum interval between successive heat stimulations of the same skin site was one min.

*Test drugs*

The test drugs were (±)-quinpirole (a dopamine $D_2$ receptor agonist; Sigma-Aldrich Co. St. Louis MO, USA), *S*-(−) eticlopride (a dopamine $D_2$ receptor antagonist; Sigma), naloxone methiodide (an opioid receptor antagonist that only poorly spreads to the central nervous system following peripheral administration; Sigma), methysergide maleate salt (a 5-HT receptor antagonist that is not selective for 5-HT receptors subtypes; Sigma), atipamezole (an $\alpha_2$-adrenoceptor antagonist that is not selective for $\alpha_2$-adrenoceptor subtypes and that does not bind to the 5-$HT_{1A}$ receptor, unlike many other $\alpha_2$-adrenoceptor antagonists; OrionPharma, Espoo, Finland; Pertovaara et al., 2005), glutamate (Sigma), and saline placebo.

*Course of the behavioral study*

The test groups in the behavioral study are listed in Table 1. When assessing pain-related behavior, the assessments were performed before and at various time points following injections of the test drugs. When attempting to reverse the effects of striatal drug injections, various receptor antagonists were administered intrathecally 15 min before the striatal injection. Mechanical and heat sensitivity were determined in separate sessions. Each animal participated in one to three behavioral testing sessions and the interval between testing sessions in the same animal was at least 2 days. Before using the same animal again, the response to monofilaments was determined to exclude any long-term effects by preceding drug administrations. After the behavioral session, the animals participated in a final electrophysiological session (see below).

*Electrophysiological recordings of neurons in the rostroventromedial medulla*

To determine the potential role of various types of rostroventromedial medullary (RVM) neurons to the antihypersensitive effect induced by striatal quinpirole, the effect of striatal drug injections on discharge rates of RVM neurons was assessed. Recordings were performed only in animals with a behaviorally verified neuropathy about 3–4 weeks following the induction of SNI. Animals used in electrophysiological recordings had participated in behavioral testing sessions 2–5 days earlier (see above). At the beginning of the recording session, anesthesia was induced by administering 60 mg/kg of sodium pentobarbital i.p. Following induction of anesthesia, the animal was placed in a standard stereotaxic frame according to

Table 1
Treatment groups in the behavioral study

| Treatment group | *n* | Treatment received |
|---|---|---|
| Quin-3 | 7 | Quinpirole 3 μg IC |
| Quin-10 | 22 | Quinpirole 10 μg IC |
| Quin-20 | 8 | Quinpirole 20 μg IC |
| Quin-30 | 9 | Quinpirole 30 μg IC |
| Quin-10 contra | 4 | Quinpirole 10 μg IC contralaterally |
| Unoper-Quin-10 | 4 | Unoperated control, Quinpirole 10 μg IC |
| Sal | 6 | Physiological saline IC |
| Quin-10 (Heat) | 4 | Quinpirole 10 μg IC (tail-flick and plantar heat tests) |
| Eti+Quin | 4 | Eticlopride 3 μg IT+Quinpirole 10 μg IC |
| Nal+Quin | 3 | Quinpirole 10 μg IC+Naloxone methiodide 10 μg IT |
| Met+Quin | 5 | Methysergide 10 μg IT+Quinpirole 10 μg IC |
| Ati+Quin | 4 | Atipamezole 5 μg IT+Quinpirole 10 μg IC |
| Eti IT | 4 | Eticlopride 3 μg IT |
| Nal IT | 4 | Naloxone methiodide 10 μg IT |
| Met IT | 4 | Methysergide 10 μg IT |
| Ati IT | 4 | Atipamezole 5 μg IT |
| Quin-30 IT | 4 | Quinpirole 30 μg IT |
| Quin-100 IT | 13 | Quinpirole 100 μg IT |

IC=intracranial (intrastriatal); IT=intrathecal.

the atlas of Paxinos and Watson (1998) and anesthesia was continued by administering sodium pentobarbital at the dose of 15–20 mg/kg/h. The level of anesthesia was frequently monitored by assessing the size of the pupils, general muscle tone and reflex responses to noxious pinching. Supplemental doses of sodium pentobarbital were given as required. The rats were spontaneously breathing and the body temperature was maintained within a physiological range with a warming blanket. The peripheral perfusion was checked by examining the color of the ears and extremities.

With the rat secured in a stereotaxic frame, a hole was drilled in the skull for placement of a stainless steel lacquer-coated tungsten microelectrode (with tip impedance of 5–7 MΩ at 1 kHz) into the RVM (AP: −2.3 mm, ML: 0.0 mm, DV: 7.8–9.8 mm; Paxinos and Watson, 1998). The signal was amplified and filtered using standard techniques. Data sampling was performed with a computer connected to a CED Micro 1401 interface and using Spike 2 software (Cambridge Electronic Design, Cambridge, UK). Actual recordings did not start until the animal was under light anesthesia; i.e., the animals gave a brief withdrawal response to noxious pinch, but the pinch did not produce any longer lasting motor activity, nor did the animals have spontaneous limb movements. Before striatal drug administrations, neurons were classified based on their response to noxious pinch of the tail with a hemostatic clamp. This stimulus was painful when applied to the finger of the experimenters. Neurons giving excitatory responses to pinch were considered ON-like ones, those giving inhibitory responses were considered OFF-like neurons and neurons giving no or only a negligible response to pinch were considered NEUTRAL-like neurons. In the present study, we defined that a pinch-evoked change in the discharge rate had to be over 15% to be considered as an excitatory or inhibitory response. Our classification scheme of medullary neurons was modified from that described by Fields and his co-workers (2006). Since we did not verify whether pinch-evoked responses of RVM neurons were associated with spinal reflex responses as in the original classification scheme (Fields et al., 2006), we use the terms ON-like or OFF-like neuron, instead of ON- or OFF-neuron. Our previous results suggest, however, that there is practically no difference in the classification of RVM neurons whether or not spinal reflex responses are concurrently measured in lightly anesthetized animals (Pertovaara et al., 2001).

After identification and classification of the RVM neuron, its spontaneous activity was measured for 1–3 min. Then, quinpirole (QUIN) at the dose 10 μg, glutamate (GLU) at the dose of 50 nmol or physiological saline was injected into the striatum. Neuronal discharge rate was then recorded up to 30 min post drug or saline administration. Table 2 shows the distribution of cells recorded in different drug conditions. It should be noted that in some cases it was possible to record simultaneously more than one RVM neuron. In general, quinpirole was injected once during the recording and glutamate or saline injections were performed 1–3 times in the same session at intervals of about an hour. In the final analysis, the mean discharge rate following striatal injection of drug or saline was compared in per cent with the corresponding mean discharge rate measured following the injection. In these comparisons, the discharge rate 100% represents the discharge rate prior to injection and a discharge rate >100% represents an increase and a rate <100% a decrease in the discharge rate by striatal injections. With quinpirole and saline, post-injection discharge rate was measured 15 min following striatal injections, while with glutamate injections post-injection discharge rate was measured 1–3 min following striatal injections. These time points were selected based on accompanying behavioral findings and on pharmacokinetic properties of drugs. These time points also proved to reflect maximum effects induced by striatal injections in the present study. At the end of the session, the animals were given a lethal dose of sodium pentobarbital and the brains were removed. After fixing the brains in 4% paraformaldehyde, they were sectioned with a vibratome for verification of the recording and injection sites. Representative slices were stained with formol–Thionin technique (Donovick, 1974).

Table 2
The distribution of RVM neurons recorded following the administration of quinpirole, glutamate or saline into the striatum ipsilateral to the nerve-injured limb

| Test drug | Type of cell recorded | Number of cells recorded |
|---|---|---|
| Quinpirole 10 μg | On | 22 |
| Quinpirole 10 μg | Off | 15 |
| Quinpirole 10 μg | Neutral | 10 |
| Glutamate 50 nmol | On | 16 |
| Glutamate 50 nmol | Off | 50 |
| Physiological saline | Off | 13 |

*Assessment of the innocuous H-reflex in control animals*

To exclude the possibility that the attenuation of pain-related responses by striatal quinpirole was due to suppression of motor behavior, we determined the innocuous H-reflex in the hind limb ipsilateral to the striatal injections. Due to SNI-induced denervation of the hind limb, it was not possible to determine the H-reflex in the neuropathic hind limb. Therefore, the H-reflex was assessed in a group of unoperated control animals using a technique that was slightly modified from that described earlier (Gozariu et al., 1998). The reflex measurements were performed under light sodium pentobarbital anesthesia (25 mg/kg i.p. followed by supplemental administration of 15–20 mg/kg/h). Needle electrodes were transcutaneously applied to the ankle of the hind limb for stimulation of the tibial nerve (constant current 0.1 ms pulses of 0.5 Hz). The amplitude of the tibial nerve stimulation was adjusted to give a submaximal H-reflex response. A concentric bipolar electrode was applied to the plantar muscles of the hind paw for recording of electromyographic (EMG) response. In addition to the monosynaptic H-reflex response, also the M-response (caused by a direct activation of α-motoneurons and a consequent activation of the effector muscle) was observed to verify stability of the stimulation and recording conditions. The amplitude of the H-reflex and M-response was determined by averaging 10–20 consecutive responses to tibial nerve stimulation prior to and 15 min after striatal administration of saline ($n=3$) or quinpirole at the dose of 10 μg ($n=4$). Only one

drug condition was studied in each animal. At the end of the session, the animals were given a lethal dose of sodium pentobarbital and the brains were removed for verification of injection sites as described above.

*Statistical analyses*

Statistical analysis was performed using one-way or two-way analysis of variance (ANOVA) followed by Tukey's test or the Student's *t*-test (comparisons between two groups). Results have been presented as mean ± S.E.M. $p < 0.05$ was considered to represent a significant difference.

## Results

*Mechanical hypersensitivity and its attenuation by striatal administration of quinpirole*

By 2–4 weeks post surgery, rats with SNI had developed marked hypersensitivity to innocuous mechanical stimulation of the skin area innervated by the sural nerve ipsilateral to the SNI. This is indicated by the finding that the pain scores to von Frey hair stimulation of the lateral plantar surface of the paw were significantly higher in the SNI than the unoperated group ($F_{1,47} = 20.9$, $p < 0.0001$; Fig. 1A). The pain scores were significantly different between the limbs ($F_{1,47} = 7.5$, $p < 0.01$), but this side-dependent difference was seen only in the SNI group ($F_{1,47} = 7.5$, $p < 0.01$). The pain score to stimulation of the unoperated limb in the SNI group was not significantly higher than the mean pain scores to stimulation of the limbs in the unoperated group (Fig. 1A).

In the SNI group, administration of quinpirole, a dopamine $D_2$ receptor agonist, in the striatum ipsilateral to the nerve injury effectively and dose-dependently attenuated mechanical hypersensitivity ($F_{4,47} = 3.6$, $p < 0.02$; Fig. 1B), while pain scores to stimulation of the paw contralateral to the injury were not changed (Fig. 1D). Of the doses studied, 10 and 20 μg proved to be the most effective ones and their effects differed significantly from that induced by saline control (Fig. 1B). The onset of the attenuation of mechanical hypersensitivity occurred within 15 min (Fig. 2A). The antihypersensitive effect was sustained for at least 2 h, but it had completely disappeared when the animals were tested 1 or 2 days later (not shown). The pain score used in the assessment of hypersensitivity consists of withdrawal responses evoked by mechanical stimuli of varying intensity. To determine whether the antihypersensitive effect induced by striatal administration of quinpirole differentially influences responses evoked by various intensities of mechanical stimulation, we analyzed separately the frequency of the

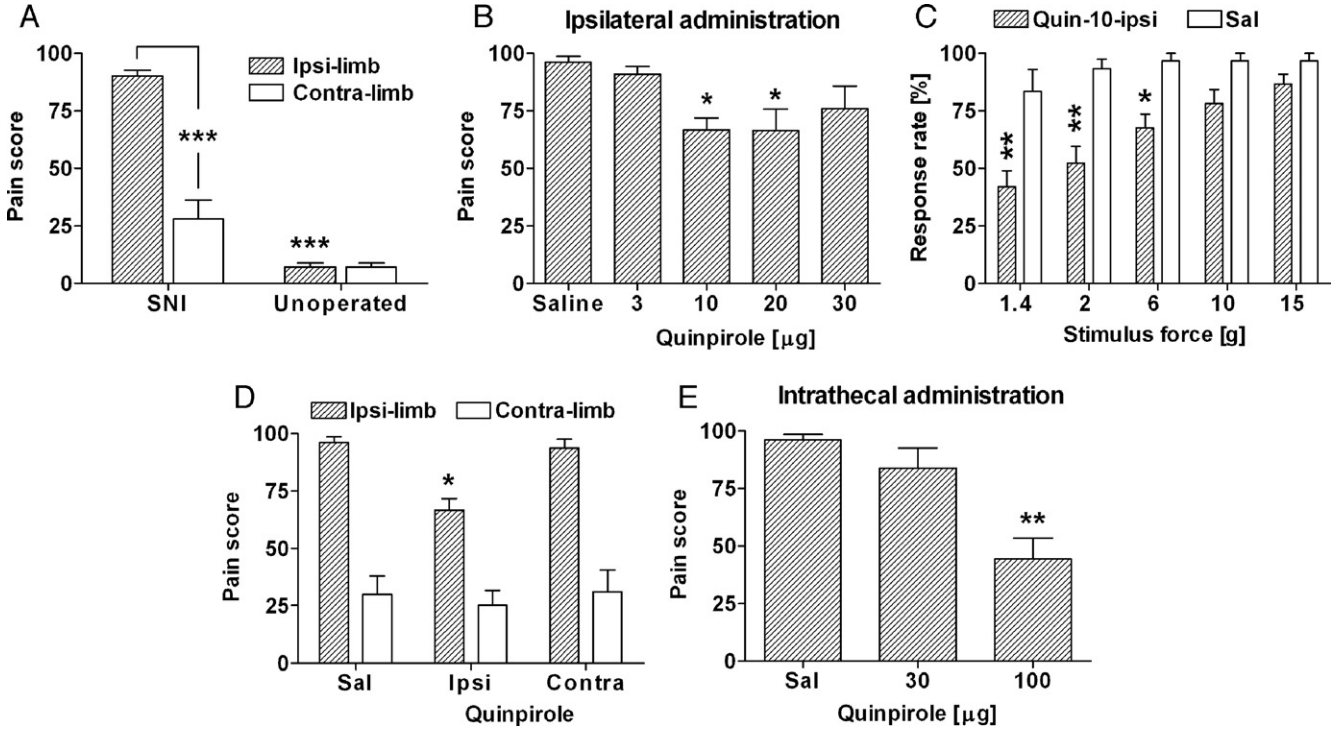


Fig. 1. Mechanical hypersensitivity assessed by determining a pain-related limb withdrawal response to repetitive mechanical stimulation of the hind paw ipsi- or contralateral to the spared nerve injury (SNI). (A) Baseline response without drug administrations. Unoperated = withdrawal responses in the left and right hind limb of unoperated controls. (B) Response of the neuropathic limb following administration of saline or quinpirole at various doses into the ipsilateral striatum. (C) Response rate of the neuropathic limb to various intensities of mechanical stimulation following administration of saline or 10 μg of quinpirole into the ipsilateral striatum. The results for these same two groups are plotted as a total pain score in graph B. (D) Mechanical hypersensitivity following administration of 10 μg of quinpirole into the striatum contralateral (Quin-contra) or ipsilateral (Quin-ipsi) to the nerve injury. Ipsi-limb = the neuropathic limb, Contra-limb = the contralateral limb. (E) Mechanical hypersensitivity following intrathecal administration of quinpirole. In each graph, pain score 100 represents the maximum pain-related response and pain score 0 represent no response. Sal = saline control. The error bars represent S.E.M. (In A, $n = 22$ in the SNI group and $n = 4$ in the unoperated group; in B–E, $n = 4$–13). In A $^*p < 0.05$ (*t*-test; reference: unless specified, the corresponding value in the SNI group). In C, $^*p < 0.05$, $^{**}p < 0.01$ (*t*-test; reference: the corresponding saline group). In B, D and E, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ (Tukey's test; reference: the corresponding saline group).

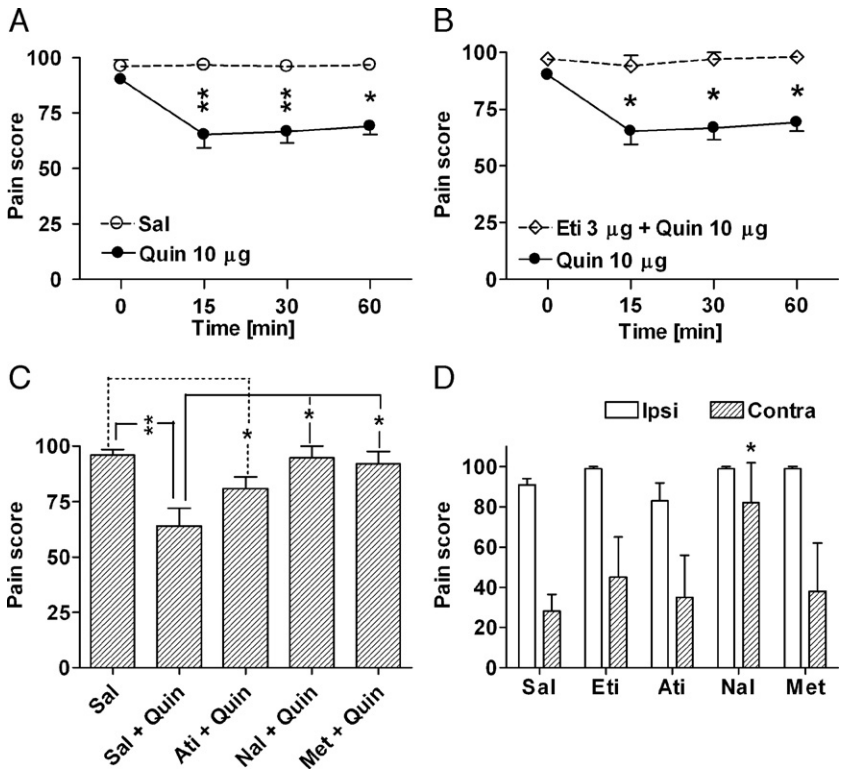


Fig. 2. Mechanical antihypersensitive effect induced by striatal administration of quinpirole (Quin) at the dose of 10 µg (A) and its attempted reversal by spinal administration of various receptor antagonists (B–C). Graph D shows the effect by intrathecal receptor antagonists alone. Sal=saline, Eti=3 µg of eticlopride (a dopamine $D_2$ receptor antagonist), Ati=5 µg of atipamezole (an $\alpha_2$-adrenoceptor antagonist), Nal=10 µg of naloxone methiodide (an opioid receptor antagonist), Met=10 µg of methysergide (a 5-HT receptor antagonist). The error bars represent S.E.M. ($n$=4–22; see Table 1 for more details). *$p<0.05$, **$p<0.01$ (In A and B, $t$-test; reference: the corresponding value in the Quin 10 µg-group. In C, Tukey's test. In D, $t$-test; reference: the corresponding pre-drug value).

withdrawal response evoked by repetitive application of each of the five monofilaments to the neuropathic limb. The rate of withdrawal responses was significantly increased with an increase in stimulus intensity from 1.4 g to 15 g ($F_{4,125}=3.8$, $p<0.01$), and ipsilateral administration of quinpirole at the dose of 10 µg into the striatum produced a significant decrease in the rate of withdrawal responses ($F_{1,125}=27.1$, $p<0.0001$), independent of the stimulus intensity ($F_{4,125}=1.3$; Fig. 1C).

Administration of 10 µg of quinpirole to the striatum contralateral to the nerve injury had no effect on mechanical hypersensitivity (Fig. 1D). In unoperated controls, striatal administration of quinpirole at the dose of 10 µg had no effect on responses to mechanical stimulation of the hind limb ipsi- or contralateral to the striatal injection ($n$=4; not shown). Striatal administration of quinpirole at the currently used doses did not produce any marked change in behavior of neuropathic or unoperated control animals, except for the antihypersensitive effect in nerve-injured animals. Following intrathecal administration, quinpirole produced a significant attenuation of mechanical hypersensitivity at the dose of 100 µg but not at the dose of 30 µg (Fig. 1E).

### *Spinal receptors involved in the antihypersensitive effect by striatal administration of quinpirole*

The attenuation of mechanical hypersensitivity induced by striatal administration of quinpirole at the dose of 10 µg (Fig. 2A) was effectively blocked by intrathecal administration of eticlopride, a dopamine $D_2$ receptor antagonist, at the dose of 3 µg ($F_{1,111}=27.06$; $p<0.0001$; Fig. 2B). Furthermore, the mechanical antihypersensitive effect induced by striatal administration of quinpirole at the dose of 10 g was reversed by intrathecal administration of 10 µg of naloxone methiodide, an opioid receptor antagonist, or 10 µg of methysergide, a non-selective serotonin receptor antagonist (Fig. 2C). In contrast, the mechanical antihypersensitive effect induced by striatal quinpirole was not reversed by intrathecal administration of 5 µg of atipamezole, an $\alpha_2$-adrenoceptor antagonist (Fig. 2C).

Intrathecal administrations of eticlopride, naloxone methiodide, atipamezole, or methysergide alone at the currently used did not suppress mechanical hypersensitivity in the neuropathic limb (Fig. 2D). Since the pain scores of the neuropathic limb were close to the maximum value already in the saline condition, it was not possible to assess reliably whether spinally administered receptor antagonists alone increased hypersensitivity in the neuropathic limb. Therefore, the possible increase of hypersensitivity was assessed in the limb contralateral to the nerve injury. While spinal administration of atipamezole, eticlopride or methysergide alone did not have a significant influence on pain scores in the contralateral limb, naloxone methiodide alone produced a significant increase in the pain scores of the contralateral limb (Fig. 2D).

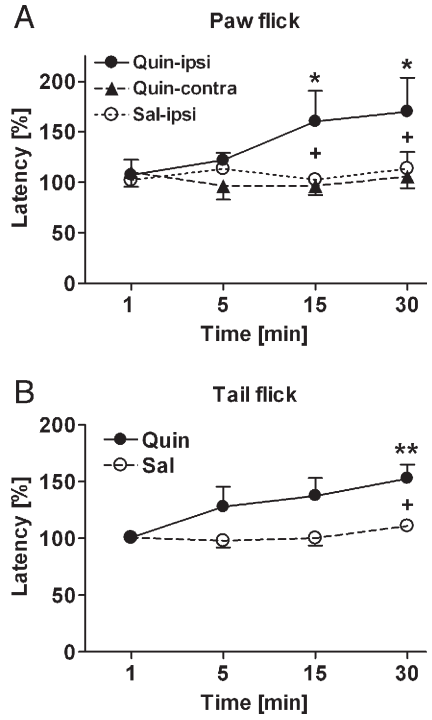


Fig. 3. Influence of striatal administration of quinpirole at the dose of 10 μg on noxious heat-evoked paw flick (A) and tail flick (B) response in animals with a spared nerve injury model of neuropathy. Quin-ipsi=withdrawal latency of the neuropathic limb ipsilateral to the striatal injection of quinpirole, Quin-contra=withdrawal latency of the limb contralateral to the nerve injury and striatal injection of quinpirole. Sal-ipsi=withdrawal latency of the neuropathic limb ipsilateral to the striatal injection of saline. Latency value 100% represents the corresponding value prior to striatal injections and time point 0 min indicates when the striatal injection was performed. The error bars represent S.E.M. ($n=4$ in each graph). *$p<0.05$, **$p<0.01$ (Tukey's test; reference: the corresponding pre-injection value), $^{+}$ ($t$-test; reference: the corresponding value in the saline group).

### *Thermal nociception*

Thermal nociception was studied only in the SNI group. Before drug administration, radiant heat applied to the sural nerve area in the hind paw induced a limb withdrawal at the latency of 10.9±0.8 s (±S.E.M., $n=4$) in the injured side and 12.5±0.4 s in the contralateral side ($p=0.05$, paired $t$-test). The mean radiant heat-induced tail-flick was induced at the latency of 7.0±0.7 s ($n=4$). When compared with the effect of saline, striatal administration of quinpirole at the dose of 10 μg produced a significant prolongation of the radiant heat-induced hind limb withdrawal latency ($F_{3,48}=9.77$, $p<0.0001$; Fig. 3A). Post hoc testing indicated that the limb withdrawal latency was increased only in the injured limb ipsilateral to the striatal administration of quinpirole (Fig. 3A). The radiant heat-induced tail-flick latency was increased following striatal administration of quinpirole, too ($F_{1,24}=14.3$; $p<0.001$; Fig. 3B).

### *Electrophysiological recordings*

#### *Effect of striatal microinjections of drugs on discharge rates of rostroventromedial medullary neurons*

To study potential role of rostroventromedial medullary (RVM) neurons in mediation of the quinpirole-induced antihypersensitive effect, we determined discharge rates of various types of RVM neurons in neuropathic rats following striatal administration of quinpirole at a dose (10 μg) that produced a mechanical antihypersensitive and thermal antinociceptive effect. Before striatal drug administrations, the mean spontaneous discharge rate of ON-like neurons giving an excitatory response to noxious peripheral stimulation was 8.1±1.8 Hz (±S.E.M., $n=22$), that of OFF-like neurons giving an inhibitory responses to noxious stimulation was 15.2±2.5 Hz ($n=15$), and that of NEUTRAL-like neurons not responding to peripheral noxious stimulation was 7.9±2 Hz ($n=10$). Quinpirole produced a significant decrease in the baseline discharge rate of ON-like neurons ($n=22$), whereas the effect of quinpirole was short of significance on baseline discharge rates of OFF-like neurons ($n=13$) or NEUTRAL neurons ($n=10$; Fig. 4A). Striatal administration of glutamate at the dose of 50 nmol had no significant effect on baseline discharge rates of ON-like ($n=16$) or OFF-like ($n=50$) neurons, nor did saline influence the baseline discharge rate of OFF-like neurons ($n=13$; Fig. 4B). Figs. 5 and 6 show the area of striatal microinjections and the medullary region in which RVM neurons were recorded.

#### *Effect of striatal quinpirole on the innocuous H-reflex*

To study potential suppression of motor behavior as a cause of antihypersensitive effect of striatal quinpirole, we assessed the innocuous H-reflex in the hind limb of lightly anesthetized animals. Since the SNI-induced denervation of the limb

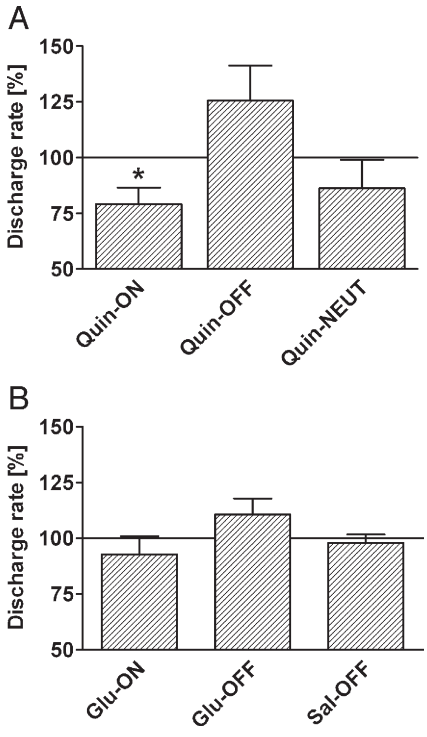


Fig. 4. Discharge rates of rostroventromedial medullary ON-, OFF- or NEUTRAL-like neurons following striatal injections of 10 μg of quinpirole (Quin), 50 nmol of glutamate (Glu) or saline (Sal). The discharge rate 100% represents the corresponding discharge rate prior to striatal injection. The error bars represent S.E.M. ($n=10$–50; see Table 2 for details). *$p<0.05$ (paired $t$-test; reference: the corresponding discharge rate prior to striatal injection).

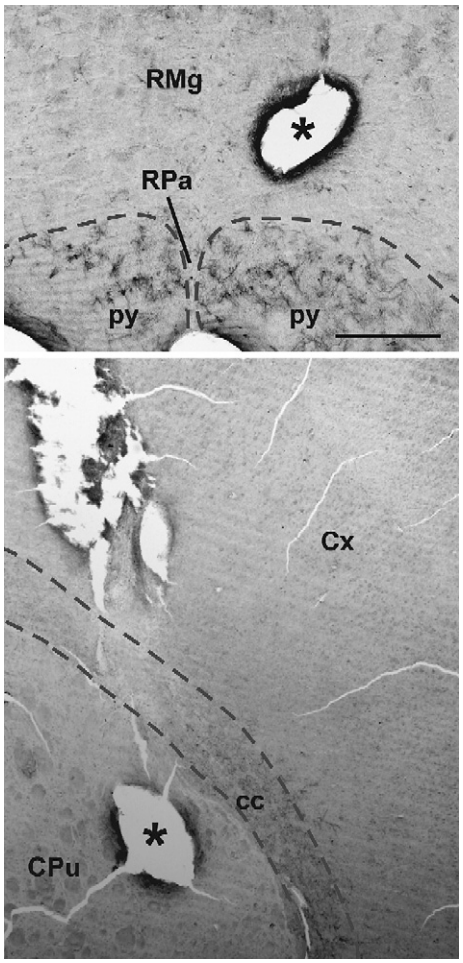


Fig. 5. Photomicrographs showing a medullary recording site (upper graph) and a striatal injection site (lower graph). The electrolytic lesion in the recording site (upper graph) and the lesion in the striatal injection site (lower graph) are shown by asterisks. In the lower graph, the lesion above the injection site indicates the site of the guide cannula in the cortex. The upper and lower part of the brain section shown in the lower graph were photographed separately and composed digitally into one photomicrograph with Adobe Photoshop 7.0.1 software. RMg=raphe magnus, RPa=raphe pallidus, py=pyramidal tract, Cx=cerebral cortex, cc=corpus callosum, CPu=caudate–putamen. The calibration bar represents 500 μm.

prevents the study of the H-reflex in the neuropathic limb, the H-reflex was studied in an intact limb. Quinpirole at a dose of 10 μg or saline control did not suppress the amplitude of the innocuous H-reflex in the hind limb ipsilateral to their striatal administration (Fig. 7).

## Discussion

The results indicate that striatal dopamine $D_2$ receptors attenuate pain-related responses in animals with peripheral neuropathy. The attenuation of pain-related responses was predominant in the neuropathic hind limb ipsilateral to the striatal injection, although nociception in the midline (tail) was also suppressed. The predominantly ipsilateral effect suggests that involvement of a midline structure, such as the RVM, may not alone explain the attenuation of a hypersensitive spinal reflex response, but an ipsilateral relay in the brainstem, such as A11, is likely to contribute to the spinal antihypersensitive effect (see further discussion below). The dose of a selective dopamine $D_2$ receptor agonist producing attenuation of hypersensitivity in neuropathic animals was not sufficient to reduce mechanical nociception in healthy control animals, indicating enhanced antinociceptive efficacy of striatal $D_2$ receptors in neuropathy. Interestingly, the dose–response curve for the antihypersensitive effect of striatal quinpirole was U-shaped. It should also be noted that since the striatal microinjection of quinpirole at the volume of 0.5 μl is likely to spread from the injection site to the adjacent cortical areas (Myers, 1966), we cannot exclude a possible contribution of cortical dopamine $D_2$ receptors to the present findings.

The antihypersensitive effect induced by striatal administration of a dopamine $D_2$ receptor agonist was not associated with any other marked changes in behavior or with a suppression of an innocuous H-reflex. It should be noted, however, that in this study the H-reflex was assessed in an intact limb. Earlier studies indicate that striatal stimulation could selectively suppress nociceptive responses in the spinal trigeminal nucleus, without an influence on the activity of trigeminal motoneurons (Belforte and Pazo, 2005) and that striatal administration of a dopamine $D_2$ receptor agonist at an antinociceptive dose did not attenuate motor behavior (Magnusson and Fisher, 2000). Although the striatum is involved in motor control circuitries, the present and earlier findings indicate that the striatum may also selectively

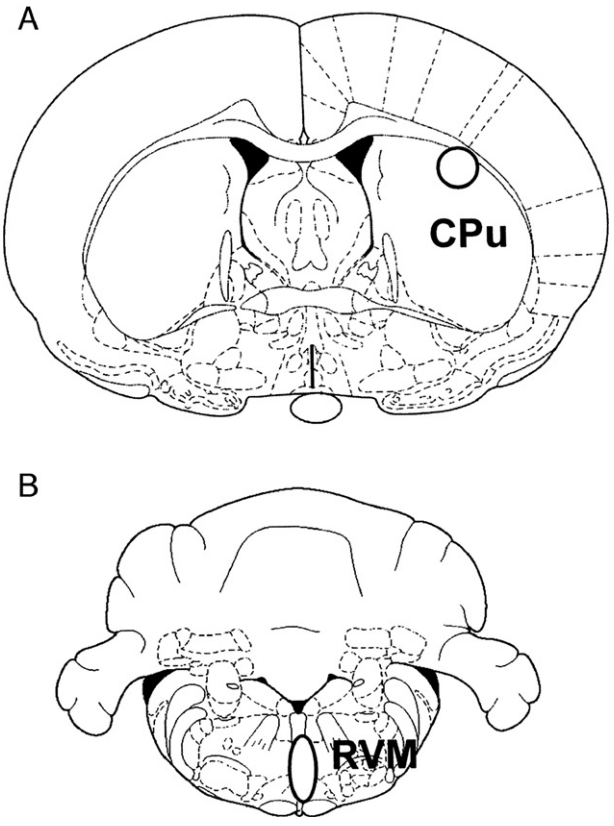


Fig. 6. (A) The extension of the striatal area in which the centers of microinjections were in the behavioral and electrophysiological experiments. CPu=caudate–putamen. (B) The extension of the medullary area in which single-unit recordings were performed. RVM=rostroventromedial medulla.

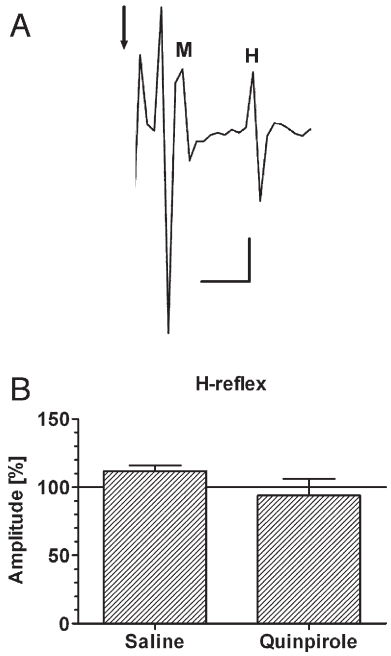


Fig. 7. (A) H-reflex and the M-response in the hind limb. The arrow indicates time of stimulation. The curve shows an average response to 10 repetitive stimuli. The horizontal calibration bar represent 4 ms and the vertical one 5 μV. (B) Amplitude of the H-reflex following injection of saline or 10 μg of quinpirole into the ipsilateral striatum. Amplitude value 100% represents the corresponding value prior to striatal injection. The error bars represent S.E.M. ($n$=3 in saline group and $n$=4 in quinpirole group).

attenuate pain-related sensory responses in neuropathic as well as control conditions.

### *Role of rostroventromedial medullary neurons in striatal modulation of pain behavior*

The rostroventromedial medulla (RVM) has an important role in descending pain modulation, and it provides the final common pathway for descending influence from various brain regions to the spinal dorsal horn (Gebhart, 2004). Moreover, the RVM is involved in relaying striatal influence on brainstem reflex blink circuits through a pathway including the superior colliculus and the raphe magnus nucleus in the RVM (Basso and Evinger, 1996). In the present study, striatal activation of dopamine $D_2$ receptors attenuated the discharge rate of the ON cell-like RVM neurons. Since ON-cells of the RVM have a pronociceptive role (Fields et al., 2006), their decreased discharge rate might contribute to the striatal dopamine $D_2$ receptor-induced attenuation of pain-related behavior in peripheral neuropathy. Increased discharge rate of OFF cell-like RVM neurons that presumably have an antinociceptive role (Fields et al., 2006) was short of significance, suggesting that the OFF-like neurons of the RVM had only a minor contribution to the striatal dopamine $D_2$ receptor-induced suppression of pain behavior. NEUTRAL-cells of the RVM, that have a less clear role in pain modulation (Fields et al., 2006), were not influenced by striatal dopamine $D_2$ receptors. Microinjection of glutamate into the dorsal striatum did not, unlike a dopamine $D_2$ receptor agonist, produce significant changes in the discharge rates of RVM cells. This finding suggests that the antinociceptive efficacy of a striatal glutamate injection is lower than that of a dopamine $D_2$ receptor agonist. However, a recent study showed that the antinociceptive effect of glutamate varies significantly with the site of microinjection within the striatum (Belforte and Pazo, 2005). The lack of glutamate effect in the present study might be explained by a non-optimal dose or site of its administration within the striatum.

### *Spinal neurotransmitter receptors involved in suppression of pain-related behavior induced by striatal dopamine $D_2$ receptors*

The mechanical antihypersensitive effect induced by striatal dopamine $D_2$ receptors was reversed by spinal administration of a dopamine $D_2$ receptor antagonist, an opioid receptor antagonist or a non-selective 5-HT receptor antagonist, but not by an $\alpha_2$-adrenoceptor antagonist. The reversal of the antihypersensitive effect by spinal administration of a dopamine $D_2$ receptor antagonist indicates that striatal dopamine $D_2$ receptors activated a dopaminergic nucleus with descending axonal projections acting on spinal dopamine $D_2$ receptors. This is in line with previous results indicating that activation of the A11, a dopaminergic brainstem nucleus, induces a spinal antinociceptive effect due to action on spinal dopamine $D_2$ receptors (Fleetwood-Walker et al., 1988) and that spinal administration of a dopamine $D_2$ receptor agonist has an antinociceptive action (Jensen and Smith, 1982; Jensen and Yaksh, 1984; Tamae et al., 2005). The present results indicate that the dose of the dopamine $D_2$ receptor agonist needed to attenuate neuropathic hypersensitivity is an order of magnitude higher following spinal than striatal administration.

The raphe magnus nucleus in the RVM provides a major source of serotoninergic innervation to the spinal cord (Kwiat and Basbaum, 1992). A number of previous studies have demonstrated that raphe-spinal serotoninergic neurons are involved in the modulation of pain-related responses (e.g., Rivot et al., 1984). It should be noted, however, that the pain modulatory role of 5-HT appears to be complex and the direction of its modulatory effect in the spinal dorsal horn is influenced by many factors such as the type of 5-HT receptor and the pathophysiological condition. Spinal 5-$HT_3$ receptors have a pronociceptive role in neuropathic animals (Suzuki et al., 2004), whereas spinal 5-$HT_{1A}$ receptors predominantly suppress pain-related responses in neuropathic and control conditions (el-Yassir and Fleetwood-Walker, 1990; Lin et al., 1996; Liu et al., 2002; Wei and Pertovaara, 2006). These previous results suggest that the reversal of the antihypersensitive effect of striatal dopamine $D_2$ receptors by a non-selective 5-HT receptor antagonist in the present study was predominantly due to the blocking of raphe-spinal serotoninergic action on spinal 5-$HT_{1A}$ receptors.

Endogenous opioids acting on spinal opioid receptors have an important role in the regulation of spinal nociception (Fields et al., 2006; Dickenson and Kieffer, 2006). In the present study, spinal administration of an opioid receptor antagonist completely reversed the antihypersensitive effect induced by striatal administration of a dopamine $D_2$ receptor agonist. At the dose

used, however, the opioid receptor antagonist alone enhanced hypersensitivity. Therefore, opioid receptor antagonist-induced reversal in the present study does not allow the conclusion that spinal opioid receptors mediate the antihypersensitive effect of striatal dopamine $D_2$ receptors.

The attempt to reverse the antihypersensitive effect of striatal dopamine $D_2$ receptors by spinal administration of an $\alpha_2$-adrenoceptor antagonist was short of significance. Thus, while earlier studies indicate that the noradrenergic system has a significant role in the feedback inhibition of sustained pain (Pertovaara, 2006), the present findings suggest that descending noradrenergic pathways do not play a critical role in striatal modulation of hypersensitivity in the SNI model of neuropathy. It should also be noted that striatal dopamine $D_2$ receptors may influence pain-related responses not only through descending influence on sensory pathways, as in the present study, but possibly also through supraspinal action on non-sensory factors. For example, the association between striatal dopamine $D_2$ binding and the subject's response criterion towards pain suggests that modulation of motivational factors by striatal dopamine $D_2$ receptors may contribute to the subject's experience of pain (Pertovaara et al., 2004).

## Conclusions

In line with earlier results showing that striatal dopamine $D_2$ receptors are involved in pain regulation in non-neuropathic conditions (see Introduction for references), the present results indicate that striatal dopamine $D_2$ receptors influence pain-related responses also in neuropathic conditions. The striatal influence on neuropathic hypersensitivity involves suppression of pronociceptive ON-like cells in the RVM, activation of descending serotoninergic pathways acting on spinal 5-HT receptors, and activation of descending dopaminergic pathways acting on spinal dopamine $D_2$ receptors. The involvement of striatal dopamine $D_2$ receptors in the regulation of neuropathic hypersensitivity is in line with the hypothesis that a dysfunction of the nigrostriatal dopaminergic system, such as in Parkinson's disease, may increase pain sensitivity in patients with as well as without peripheral neuropathy (Scherder et al., 2005; Wasner and Deuschl, 2006).

## Acknowledgments

This study was supported by the Academy of Finland and the Sigrid Jusélius Foundation, Helsinki, Finland.

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